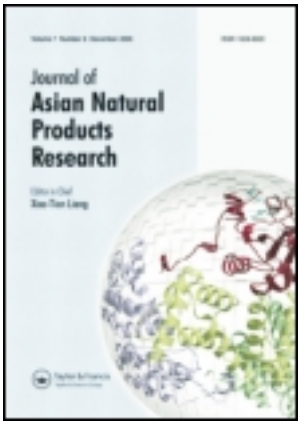

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

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Available online: 30 Mar 2011

To cite this article: Xiu-Lan Xin, Xun Cui, Changyuan Wang, Bao-Jing Zhang, Xiao-Chi Ma, Shan-Shan Huang, Sha Deng, Yan Tian, Hou-Li Zhang & Mei-Hua Yang (2011): Microbial transformation of deoxyandrographolide by *Fusarium graminearum* AS 3.4598, Journal of Asian Natural Products Research, 13:04, 350-355

To link to this article: http://dx.doi.org/10.1080/10286020.2011.558009

Journal of Asian Natural Products Research
Vol. 13, No. 4, April 2011, 350–355

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(*Received 21 December 2010; final version received 24 January 2011*)

Biotransformation of deoxyandrographolide (**1**) by *Fusarium graminearum* AS 3.4598 was investigated in this paper. And five transformed products of **1** by *F. graminearum* AS 3.4598 were obtained. Their chemical structures were characterized as 3-*oxo*-8α,17β-epoxy-14-deoxyandrographolide (**2**), 3-*oxo*-14-deoxyandrographolide (**3**), 3-*oxo*-17,19-dihydroxyl-8,13-*ent*-labdadien-15,16-olide (**4**), 1β-hydroxyl-14-deoxyandrographolide (**5**), and 7β-hydroxyl-14-deoxyandrographolide (**6**) by spectral methods including 2D NMR. Among them, products **2**, **4**, and **5** are new.

Keywords: microbial transformation; deoxyandrographolide; hydroxylation; *ent*-labdane

1. Introduction

Chuanxinlian is a common herb from dried aerial parts of *Andrographis paniculata* (Burm. f.) Nees distributed in China and India [1]. Its stems and leaves are widely used as antivirin, antitumor, and anti-inflammatory and antipyretic drug [2,3]. The principal active constituents of *A. paniculata* are the ent-labdane diterpenoids such as andrographolide, deoxyandrographolide, and dehydroandrographolide. They usually have the characteristic A/B *cis* junctures with α-alkylidene-γ-butyrolactone moiety and α-hydroxyl group at C-3 position. They have anticancer [4] and hypotensive activities [5]. In recent years, the structure–activity relationships of andrographolide analogs in biological activities of α-glucosidase inhibitory [6] and cytotoxic agents [7] have been reported, which indicates that they have potent activities of anticancer.

Microbial transformation is an important approach for modifying the bioactive substrates by the enzymes of microorganisms, with the advantages of high stereo- or regio-selectivity, as well as mild reaction conditions over chemical synthesis. In addition, microbial transformation is also regarded as a useful *in vitro* model to identify the metabolites *in vivo* [8]. In recent years, microbial biotransformation of andrographolide and dehydroandrographolide, as the unique and inexpensive resource, has been widely reported to modify their structures and obtain some new chemical entities [9–11].

In this paper, the biotransformation of deoxyandrographolide (**1**) by *Fusarium graminearum* AS 3.4598 was carried out

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.558009
http://www.informaworld.com

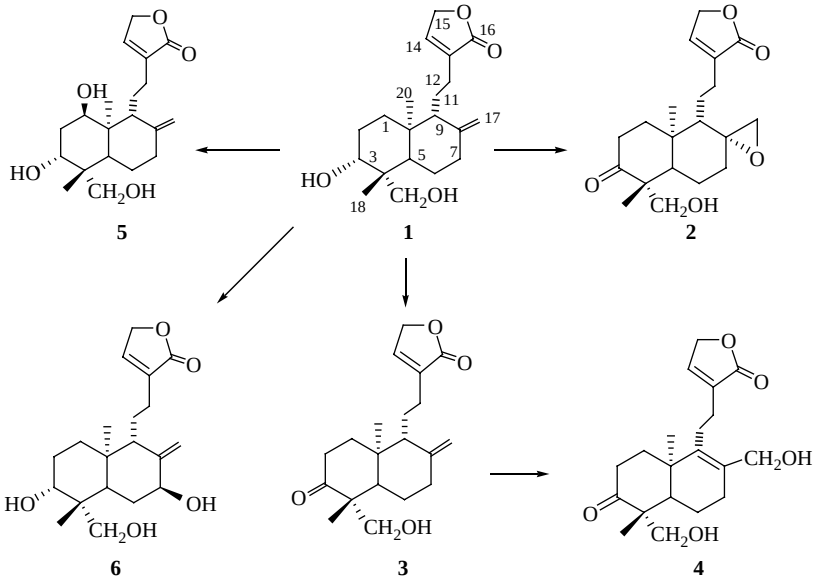

Figure 1. A possible biotransformation pathway of **1** by *F. graminearum* AS 3.4598.

with the aim of improving its activities and solubility. Five transformed products were isolated, and their structures were elucidated on the basis of extensive spectral data including HMQC, HMBC, and NOESY. Among them, products **2**, **4**, and **5** were new compounds.

2. Results and discussion

In the present study, compound **1** was added into the 48-h-old cultures of *F. graminearum* AS 3.4598 and continued to incubate for an additional 5 days. And a blank culture control and a substrate control were carried out as described above. HPLC analyses showed that **1** was stable in the culture medium. In the preparative-scale biotransformation, a total amount of 400 mg of substrate was added into the culture liquid of *F. graminearum* AS 3.4598. After 5 days of incubation, five more polar products were isolated by chromatographic methods. Using ^{1}H, ^{13}C, and 2D NMR techniques, their structures were characterized as 3-*oxo*-8α,17β-epoxy-14-deoxyandrographolide (**2**), 3-*oxo*-14-deoxyandrographolide (**3**), 3-*oxo*-17,19-dihydroxyl-8,13-*ent*-labdadien-15,16-olide (**4**), 1β-hydroxyl-14-deoxyandrographolide (**5**), and 7β-hydroxyl-14-deoxyandrographolide (**6**), respectively (Figure 1). Among them, compounds **2**, **4**, and **5** are new products. And known products **3** and **6** were in agreement with those reported in the literature [9,10].

Compound **2** was isolated as a white powder with $[\alpha]_D^{22}$ +18.6 ($c = 0.08$, MeOH). Its HR-MS provided a *quasi*-molecular ion $[M + Na]^+$ at *m/z* 371.1825, suggesting the molecular formula of $C_{20}H_{28}O_5$. In the ^{1}H NMR spectrum, two quaternary methyl signals at δ 1.05 and 1.48 were observed, respectively. In DEPT and HMQC spectra, two olefin protons disappeared and the presence of a new oxygen-bearing CH_2 at δ 51.0 was observed. These evidences indicated that an epoxy ring would be substituted in the molecule of **2**. Meantime, an additional carbonyl group (δ 214.0) was also observed. In addition, the proton signal of δ 2.85 had the HMBC correlations with carbon signals at δ 58.7 (C-8), 53.5 (C-9),

and 37.0 (C-7), respectively. And the carbon signal of δ 58.7 had the long-range correlations with H_a-11 (δ 1.39), H-9 (δ 1.58), and H_b-7 (δ 1.40), all of which suggested the epoxidation of C-8, 17 in the chemical structure of **2**. In addition, comparing with **1** [11], the carbon signals at δ 29.0 (C-2) and δ 43.3 (C-4) shifted upfield to δ 36.1 and 55.2, respectively. And the carbon signal of δ 214.0 had the HMBC correlations with H-1, H-2, and H-18, proving that a ketone group should be at C-3. In the NOESY experiment, the proton signal of δ 2.85 had the NOE enhancement with H-9, suggesting H-17 should be in β-configuration. On the basis of the above analysis, compound **2** was characterized as 3-*oxo*-8α,17β-epoxy-14-deoxyandrographolide. All the ^{1}H and ^{13}C NMR spectral data were unambiguously assigned by the 2D NMR technology.

Compound **4** was afforded as a white powder (MeOH) with $[\alpha]_D^{22}$ −26.3 ($c = 0.15$, MeOH). It showed a molecular formula of $C_{20}H_{28}O_5$ by HR-ESI-MS ($[M + Na]^+$, found $m/z = 371.1837$). By comparing with **1**, the ^{13}C NMR and DEPT spectra of **4** showed disappearance of a CH_2 signal (δ 110.1) and the appearance of quaternary carbon (δ 140.7) and CH_2 (δ 62.7), suggesting that compound **4** was a hydroxylated product of **1**, with the rearrangement of a double bond at C-8 and C-17. In the HMBC spectrum, the carbon signal (δ 140.7) had correlated with the protons of H-1, H-8, H-11, and H-20. Meanwhile, the carbon signal (δ 133.8) had the HMBC correlations with H-6, H-7, and H-17, all of which indicating that a double bond should be at C-8 and C-9 positions. The oxygen-bearing CH_2 signals (δ 4.55 and 4.34) had the long-range correlations with C-7, C-8, and C-9, indicating that a hydroxymethyl group should be located at C-8. In addition, the carbon signal of δ 214.9 had the HMBC correlations with H-1, H-18, and H-19, proving that a ketone group should be at C-3. On the basis of the above analysis, compound **4** was elucidated as 3-*oxo*-17,19-dihydroxyl-8,13-*ent*-labdadien-15,16-olide.

Compound **5** was obtained as a white powder, and HR-MS of **5** showed an $[M + Na]^+$ ion peak at *m/z* 373.1981, indicating the molecular formula of $C_{20}H_{30}O_5$. In the ^{13}C NMR spectrum, an additional carbon signal (δ 72.2) was observed. In the HMBC spectrum, the carbon signal (δ 72.2) correlated with H-2 (δ 2.42), H-20 (δ 0.89), H-3 (δ 4.58), and H-5 (δ 2.07). In addition, the ^{1}H–^{1}H COSY spectrum showed that H-2 (δ 2.42) had correlations with H-1 (δ 4.06), suggesting a hydroxyl group to be introduced at C-1. In the NOESY spectrum, H-1 (δ 4.06) had the NOE enhancements with Me-20 (δ 0.89), suggesting that the 1-OH should be in β-configuration. On the basis of the above analysis, compound **5** was determined as 1β-hydroxyl-14-deoxyandrographolide.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer 243B polarimeter. UV spectra were detected on a YV-1091 UV–vis spectrophotometer. IR spectra were obtained on an Avatar 360 FT-TR spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) in pyridine-d_5 with TMS as an internal standard. ESI-MS data were obtained by Bruker APEX IV FT-MS. The HPLC equipment was an Ultimate 3000 HPLC system with pump, autosampler, column compartment, and photodiode array detector. The HPLC data were recorded by CHROMELEON chromatography management systems. Silica gel (200–300 mesh) was purchased from Qingdao Marine Chemical Group, Qingdao, China. All solvents including ethyl acetate, petroleum ether (60–90°C), and acetone are A.R. grade and were

Table 1. ^{1}H NMR spectral data of compounds **2**, **4**, and **5** (pyridine-d_5, 500 MHz, δ in ppm, J in Hz).

H	**2**	**4**	**5**
1a	1.96 m	2.14 m	4.06 (t, 5.2)
b	1.50 m	1.70 m	
2a	2.84 m	2.80 m	2.42 m
b	2.37 m	2.44 m	2.36 m
3	–	–	4.58 (dd, 13.0, 2.5)
5	1.59 (dd, 14.5, 5.5)	1.79 br d (12.1)	2.07 br d (12.5)
6a	1.75 m	2.65 m	2.60 m
b	1.60 m	2.33 m	2.38 m
7a	1.84 m	2.54 m	2.40 m
b	1.40 m	2.48 m	2.01 m
9	1.58 (t, 4.5)	–	2.65 (t, 4.5)
11	1.39 m	2.52 m	2.05 m
	1.49 m	2.29 m	1.70 m
12	2.35 m	2.25 m	1.90 m
	2.50 m	2.59 m	1.45 m
14	7.19 br s	7.19 br s	7.19 br s
15	4.68 (d, 1.5)	4.72 br s	4.62 (d, 2.0)
	4.72 (br d, 2.0)		
17a	2.85 (d, 4.0)	4.55 br s	4.99 (d, 4.0)
b	2.53 (d, 4.0)	4.34 br s	4.85 (d, 4.0)
18	1.48 (3H, s)	1.45 (3H, s)	1.49 (3H, s)
19	4.25 (d, 11.0)	4.29 (d, 11.0)	4.54 (d, 11.0)
	3.84 (d, 11.0)	3.84 (d, 11.0)	3.71 (d, 11.0)
20	1.05 (3H, s)	1.28 (3H, s)	0.89 (3H, s)

obtained from Beijing Chemical Reagents Company (Beijing, China). Methanol and acetonitrile used for HPLC are chromatographic grade (Merck, Darmstadt, Germany). Deoxyandrographolide (**1**) was isolated from *A. paniculata* by the authors. The purity was above 98% determined by HPLC.

3.2 Microorganism

Alternaria alternata AS 3.577, *Alternaria alternata* AS 3.4578, *Alternaria longipes* AS 3.2875, *Curvularia lunata* AS 3.4381, *Cunninghamella blakesleana* lender AS 3.970, *Cunninghamella elegans* AS 3.1207, *Cunninghamella elegans* AS 3.2028, *F. graminearum* AS 3.4598, *Mucor subtilissimus* AS 3.2454, *Mucor spinosus* AS 3.3450, *Mucor spinosus* AS 3.2450, *Mucor spinosus* AS 3.3447, *Mucor subtilissimus* AS 3.2456, *Mucor polymorphosporus* AS 3.3443, *Penicillium melinii* AS 3.4474, *Penicillium janthinellum* AS 3.510, *Rhizopus stolonifer* AS 3.3463, *Rhizopus stolonifer* AS 3.2050, *Rhizopus arrhizus* AS 3.2897, *Syncephalastrum racemosum* AS 3.264, and *Trichoderma viride* AS 3.2942 were purchased from China General Microbiological Culture Collection Center in Beijing, China.

3.3 Culture medium

All culture and biotransformation experiments using filamentous fungi were carried out in potato medium, which consists of 200 g of peeled potato extract, 20 g of glucose, and 1000 ml of distilled water [12]. The culture medium was autoclaved at 121°C and 1.06 kg/cm^2 for 30 min.

3.4 Culture and biotransformation

Preparative-scale biotransformation of deoxyandrographolide by *F. graminearum* AS 3.4598 was carried out in a 1000-ml

Table 2. ^{13}C NMR spectral data of compounds **2**, **4**, and **5** (pyridine-d_5, 125 MHz, δ in ppm).

^{13}C NMR	**2**	**4**	**5**
1	38.5 t	36.5 t	72.2 d
2	36.1 t	36.5 t	37.4 t
3	214.0 s	214.9 s	75.0 d
4	55.2 s	54.8 s	43.8 s
5	56.8 d	53.8 d	49.3 d
6	23.2 t	30.1 t	25.0 t
7	37.0 t	28.5 t	39.1 t
8	58.7 s	133.8 s	149.5 s
9	53.5 d	140.7 s	49.9 d
10	40.5 s	39.4 s	43.8 s
11	20.9 t	26.4 t	22.1 t
12	27.8 t	21.9 t	25.1 t
13	134.2 s	134.2 s	134.7 s
14	145.9 d	145.8 d	145.5 d
15	71.0 t	71.1 t	70.9 t
16	175.0 s	174.9 s	175.1 s
17	51.0 t	62.7 t	107.7 t
18	21.7 q	20.9 q	24.2 q
19	65.6 t	65.5 t	64.6 t
20	15.2 q	20.6 q	16.3 q

Erlenmeyer flask. The flasks were placed on the rotary shakers, operating at 180 rpm at 28°C. After 48 h of pre-culture, the substrate (20 mg) in 1 ml acetone was added to the 400 ml medium. Finally, 400 mg of **1** was used. The incubation was continued under the above conditions for 5 additional days. The culture was filtered and the filtrate was extracted using EtOAc for three times. The organic phase was collected and concentrated to dryness at 35°C *in vacuo*.

The yellow extract (1.0 g) was applied to silica gel column and eluted with petroleum ether–acetone (in a gradient manner from 100:3 (v/v) to 1:1, at a flow rate of 1.5 ml/min). Fractions were monitored by HPLC-DAD and finally five transformed products including compounds **2** (19.2 mg, 4.8% yield), **3** (10 mg, 2.5% yield), **4** (4 mg, 1% yield), **5** (5.2 mg, 1.3% yield), and **6** (14 mg, 3.5% yield) were isolated.

*3.4.1 3-Oxo-8α,17β-epoxy-14-deoxyandrographolide (**2**)*

A white powder (acetone); mp 144–145°C; $[\alpha]_D^{22}$ +18.6 ($c = 0.08$, MeOH); UV λ_{max} MeOH: 215 nm; IR (KBr) ν_{max} (cm^{-1}): 3460, 2910, 1759, 1705, 1452, 1052. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-ESI-MS: *m/z* 371.1825 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_5Na$, 371.1829).

*3.4.2 3-Oxo-17,19-dihydroxyl-8,13-ent-labdadien-15,16-olide (**4**)*

A white powder (MeOH); $[\alpha]_D^{22}$ −26.3 ($c = 0.15$, MeOH); UV λ_{max} MeOH: 214 nm; IR (KBr) ν_{max} (cm^{-1}): 3256, 1750, 1075, 986. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-ESI-MS: *m/z* 371.1837 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_5Na$, 371.1829).

*3.4.3 1β-Hydroxyl-14-deoxyandrographolide (**5**)*

A white powder (MeOH); mp 203–204°C; $[\alpha]_D^{22}$ −26.3 ($c = 0.15$, MeOH); UV λ_{max} MeOH: 216 nm; IR (KBr) ν_{max} (cm^{-1}): 3440, 2925, 1710, 1401, 1062, 911. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-ESI-MS: *m/z* 373.1981 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_5Na$, 373.1985).

Acknowledgements

We thank the National Natural Science Foundation of China (No. 81073013), the Important New Drug Research Project (2009ZX09502-025), the Science and Technology Development Common Project (KM201110858001), and the Scientific Research Base Program by the Beijing Municipal Commission of Education and PHR (IHLB) for financial support.

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